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A study of certain phases of the physiology of reproduction of farm animals

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A STUDY OF CERTAIN PHASES OF THE PHYSIOLOGY OF REPRODUCTION OF FARM ANIMALS

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by

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Part I

INTRODUCTION

If definite recommendations concerning the feeding and management of breeding males are to be made, and studies on the factors affecting the development of the testes are to be conducted, it is necessary to determine the normal course of development of the testes and the time of production, and processes concerned with the proliferation of mature, functional germ cells. Little definite information concerning these processes is available. Phillips and Andrews (1935) studied the normal development of the testes of the ram and the development of the thermo-regulatory function of the tunica dartos muscle in the same species, and, with the exception of this work, the author has found no information concerning the testicular development of the farm animals.

The purpose of this paper is to present data on the testicular development of the bull and the boar, and on certain phases of the physiology of spermatozoa, and is organized into the following sections:

I. The normal testis development of the bull.

II. The thermo-regulatory function and histological development of the tunica dartos muscle of the bull.

III. Testicular development of the boar.

IV. The thermo-regulatory function and histological development of the tunica dartos muscle of the boar.

V. The transport of spermatozoa through the uterine tubes of the ewe.

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Part II

REVIEW OF LITERATURE

I. Embryology of the Testis.

In early fetal life the ovary and the testis follow an identical course of development. It is impossible to distinguish between the two organs, and during this time the primordial sex gland is designated as a gonad. Simpkins (1928), in studying human embryos, divided them into three groups: in the first group are embryos whose gonads, if present, are in the indifferent stage; in the second group are the females; and in the third, the males. The peritoneal epithelium of first month embryos is composed of a single layer of cells with spherical nuclei and but slight variations in size. The mesonephros begins to form a slight bulge in the dorsal compartment of the body cavity of 4.2 - 5.0 mm. embryos. Slight changes occur up to the 7 mm. stage (Simpkins-1928), but following this, the gonads develop rapidly and the cells of the peritoneal epithelium proliferate at an increased rate. In 10-11 mm. embryos, certain cells of the germinative epithelium begin to arrange themselves into short, cord-like processes extending from the superficial epithelium down into the stroma, and are designated as the early sex cords. In embryos from 15-18 mm. the mesonephric tubules show retrogressive changes. The cord-like arrangement of the cells of the sex gland is more pronounced; the cells of the sex cords are larger than the cells lying between them, and genitaloid cells with large, spherical nuclei lie either within or without the cords. Sex can now be differentiated, and in the male, the sex cords and rete cords, fore-

runners of the seminiferous and rete tubules respectively, progressively differentiate. The rete cords (Wilson - 1926) converge toward the mesorchium and form the dense primordium of the rete testis, and the sex cords anastomose peripherally in loops and join in twos and threes centrally before joining with the rete.

The primordial cells of the testis or sex cords, the forerunners of the seminiferous tubules, may form early spermatogonia (Arey - 1930), but the later generation probably trace their origin to the so-called indifferent elements (cells of the sex gland epithelium) which also produce Sertoli cells. The interstitial cells (Stieve - 1927) are probably produced by the mesenchymal stroma. Reiprich (1925) in studying the testis from the fourth fetal month to the second year found that normal testicular growth occurs until the eighth or ninth lunar month. At this point regressive changes are observed in the tubules, and at the end of the tenth fetal month the lumina of the tubules are occluded, and the interstitial mass is greatly increased. After birth regression decreases and the stage eight months after birth resembles that of the eighth fetal month.

Yamada (1920) demonstrated a similar condition in the pig. The number of spermatogonia increases rapidly in 10 cm. embryos, and they continue to be produced until late in fetal life. Immediately preceding birth, the number of spermatogonia decreases, and there is noticeable degeneration. Spermatogenesis begins in the fifth post-natal month and sperm appear in small numbers at about this time.

In the pig (Patten- 1927), in man (Arey- 1930), and in other species where the testes are normally carried in the scrotum, the testis leaves its place of origin in the body. At the beginning of the third fetal month in the human (Arey) while the testes are still fairly high in the abdominal cavity, the forerunners of the vaginal sacs appear in each side of the ventral abdominal wall, and the testes lie near them without changing position until the end of the sixth fetal month. The saccae vaginalae evaginate over the pubis, through the inguinal canal, and into the scrotum. The testes descend along the same path between the seventh and ninth months, and are normally in the scrotum at birth. A few months after birth the inguinal canal normally becomes occluded and its epithelium is resorbed. The vaginal sac forms the tunica vaginalis of the testis, and the vas deferens and spermatic vessels and nerves which were carried into the scrotum with the testis and epididymis are surrounded by connective tissue and form the spermatic cord.

II. Normal Post-Natal Development of the Testis and the Production of Spermatozoa.

The development of the testis and the production of spermatozoa varies with species. Most species of fish produce spermatozoa in definite cycles, and fertilization occurs but once each year. Courrier (1921) Champy(1923) and van Oordt (1923) found that the testis of the fresh water stickleback shows seasonal variation. Spermatogenesis is at its height during the winter months. With the approach of spring there is a diminution of spermatogoneal

elements and an increase in the rate of spermatozoa formation. Mature spermatozoa are abundant until April or May and decrease after this time. Amphibia, as a general rule, have a definite annual breeding season, but this may vary as to duration and season. Champy - (1913 - 1921) and Aron (1921) found that in Triton cristatus spermatogenesis occurs shortly after the breeding season and that spermatozoa remain in the testes for many months. In Cryptobranchus alleganiensis (McGregor - 1899) spermatogenesis occurs in a wave from the posterior to the anterior end of the testis in July and mature spermatozoa are produced in the latter part of August to fertilize the eggs of the female in August and early September.

The majority of reptilian reproduction is seasonal. The lizard (Reiss- 1923) shows active spermatogenesis in the fall after the elimination of spermatozoa, and spermatids are present in large numbers. The spermatids remain throughout the winter, and spermatozoa are produced the following April and May.

The testes of birds in the wild state on the Northern Hemisphere show a seasonal period of activity with at least one if not several waves of spermatogenic activity. Some tropical species produce spermatozoa constantly. The domestic fowl with the exception of the turkey and the goose, carry on continuous reproductive activity. Rowan (1928) found the testes of the Junco to show seasonal activity. Spermatogenesis occurs in the spring and is at its height in April and in May; regression begins in June or July, and the minimal function and size are reached in early November.

Among the mammalia, some species produce spermatozoa constantly (man, horse, cattle, pig, sheep, dog, guinea pig, rat, etc.) and others show strictly seasonal reproductive activities. McKenzie and Berliner (1935) find that rams produce spermatozoa throughout the entire year, but that the proportion of abnormal spermatozoa increases during the summer months (Missouri conditions) and that rams having a tendency to be of low fertility are often sterile during or following the summer season. The sexual desire is apparently highest from Sept. to Nov. and lowest during March and April. The mole (Tandler and Grosz - 1911), the hedgehog (Marshall - 1911), and the bat (Courrier - 1927) produce spermatozoa at definite seasons. Rasmussen (1917) found that in the woodchuck, during the months of August - September the testes are withdrawn into the abdominal cavity and are minimal in size. The seminiferous tubules contained Sertoli cells, spermatogonia, and non-active spermatocytes. Spermatogenic activity begins in November and December (the early hibernating period) and when the animal emerges from hibernation in March the testes are prepared for a period of active spermatogenesis. In late March or early April the testes descend into the scrotum and the tubules show an active production of spermatozoa. Late in April the tubules contain only a single row of cells and a few spermatozoa in the lumina.

Among species in which, after puberty or sexual maturity has been reached, spermatozoa are constantly produced, little data is available as to the changes occuring in the testis

from the time of birth until death. Havelock (1914) states that in the human, in temperate climates, puberty begins in the male between the fourteenth and fifteenth years, and a few years earlier in tropical climates. In farm animals it is stated that the stallion is sexually mature at from 10 - 12 months, the bull from 4-6 months, the ram from 4-5 months, and the boar from 3-5 months. Firket (1920) describes the development of the testis of the albino rat, but does not state when the first spermatozoa are produced. In the albino rat at birth the testis shows a typical sex cord. After birth the testis increases in size as the result of the active multiplication of the small epithelial germinative cells of the sex cord. In the early stage of post-natal development three of four small epithelial cells separate the two nearest primary germ cells. From the fifth to the tenth days the percentage of epithelial cells to primary germ cells increases, and degeneration of the primary germ cells takes place beginning with the fifth day.

At eight days there is one primary germ cell to eighteen small epithelial cells, and in a rat of ten to eleven days primary germ cells are rare.

Allen (1918) in studying the spermatogenesis of the albino rat found that the first spermatocytes begin to differentiate between the seventh and the tenth days after birth, and that the first mature spermatozoa appear in animals between thirty-six and forty days of age, at about the time of the descent of the testes.

Bruce (1934) in studying cell division in the sheep

reports very little mitotic activity in the testis of the sheep between birth and three months. Spermatogonial mitosis is prominent at from four to five months and increasing numbers of spermatozoa were observed by Bruce from this period onward.

Phillips and Andrews (1935) in studying the normal development of the testis of the ram present data of the changes occuring in the testis of animals from three weeks of age to sexual maturity. They found the development of the testis to be a gradual process, culminating in the production of spermatozoa. In the three weeks old individuals, the seminiferous tubules measure 36.51 micra in diameter, contain a single row of spermatogonia, a few Sertoli cells, no primary spermatocytes, and the interstitial cells are loosely arranged. As development progressed up to twenty-one weeks, primary and secondary spermatocytes appeared, spermatids developed, and, at twenty-one weeks, spermatozoa were produced; during this time the tubule size increases to 163,38 micra, Sertoli cells were easily distinguishable, and interstitial cells were orderly arranged and definitely defined.

III. Intertubular Life of the Spermatozoa in the Male Tract.

Hammond and Asdell, (1926) in studying the vitality of spermatozoa in the male tract of the rabbit, ligated the epididymis in the middle portion between the upper and lower poles. By breeding normal does, it was determined that normal fertility is maintained up to the twentieth day following the operation, and that from this period onward, a decline in fertility occurs, so that between thirty-one to

forty days only 20% of the females produced litters. These workers found motility of spermatozoa to persist up until sixty days in one case, and that motility is not an accurate measure of fertility.

Toothill and Young (1931), by means of injections of india ink into the head of the epididymis of the normal sexually inactive male guinea pig, demonstrated that the average length of time required for the passage of spermatozoa from the head of the epididymis into the anterior end of the vas deferens is between fourteen and eighteen days. These workers estimate that between twenty-five and thirty-eight days are required for the passage of spermatozoa through the epididymis in guinea pigs in which the testes and epididymis have been separated by ligature of the caput epididymis. It seems probable from this study that the constant production of liquid secretion and spermatozoa within the seminiferous tubules and, possible, also ciliary action within the ductuli efferentes are prominent factors in the passage of spermatozoa through the epididymis. Phillips (1934) found that in rams being allowed three services every third day the average time required for the passage of spermatozoa from the testes to the ejaculatory duct is 8.8 days. Young (1929) states that the strengthening of spermatozoa begins before they pass from the testes and continues after they reach the epididymis, but independently of any specific action of the epididymal secretion. Young (1931) in a series of experiments in which spermatozoa were removed from different levels of the ductus epididymis, and observations on spermatozoa con-

fined in the seminiferous tubules substantiated his earlier belief that the secretions of the epididymis do not condition the maturation of the spermatozoa. Simeone and Young (1931) found that in the guinea pig, mature, functional spermatozoa not discharged in copulations undergo regressive changes which end in their death and subsequent liquefaction within the epididymis and particularly within the vas deferens. The greatest number of non-functional and degenerating spermatozoa are found in the distal end of the vas deferens. These workers conclude: "that once full spermatozoon development is attained in a male which is not copulating frequently, there is no influence which preserves the sperm in an optimal functional condition; they age and ultimately disappear by a process of liquefaction in dissolution or in situ."

IV. Factors Influencing Spermatogenesis.

a. The Effect of the Anterior Pituitary Hormone.

Crowe, Cushing and Homans (1909) reported the first evidence of anterior pituitary-genitalia relationship. Partial pituitary ablation was followed by atrophy of the genitalia in adult dogs and a persistence of infantilism in puppies. Removal of the pituitaries of rats of any age (Smith-1927, 1930) stops further development of the gonads and causes atrophy. In immature males removal of the anterior pituary causes a decrease in testis size and failure of spermatogenesis, and if ablation is performed after maturity, the seminiferous tubules degenerate and the penis, prostate, and seminal vesicle atrophy. Smith (1932) states that implants of the anterior pituitary gland bring about complete testicular

recovery in hypophysectomized male rats of all ages. Implants in normal male rats (Aschheim and Zondek, 1926; Smith, 1926; and Smith and Engle, 1927) yield less decisive results than treatment of females; the testes increase but little in size, spermatogenesis does not appear to be stimulated, and the accessory organs are enlarged to some extent. Engle (1929), by injecting the urine of pregnant women into immature male rats, brought about a marked hypertrophy of the sex apparatus with the exception of the testis. The interstitial cells increase in size but no acceleration of spermatogenesis is observed. Brouha, Hinglais, and Simonnet (1929, 1930) caused hypertrophy of the testes and accessory organs of the rat, mouse and guinea pig with pregnancy urine and acid or neutral extracts of the anterior pituitary. The data available is not conclusive, but, in general, agreement has been reached that premature development of spermatozoa has not been demonstrated by anterior lobe extracts or prolan. In the fowl, however, extracts of the anterior pituitary have hastened spermatogenesis in the male although the accessory organs undergo little change.

b. The Effect of Testicular and Follicular Hormones on the Testis.

Bugbee and Simond (1926) demonstrated that the adult male shows few distinctly harmful effects from the injections of moderate doses of theelin. Laquere, Hart and deJongh (1926) produced degeneration in the adult testis by large doses, and Golding and Ramirez (1928) inhibited the normal growth of testicular elements with small amounts of theelin. The

testes remain small in size, spermatozoa are not produced, and testicular descent into the scrotum is inhibited. The testes grow rapidly and descend into the scrotum, following the cessation of injections. Moore and Price (1930, 1932) in studying hormone antagonism found that injections of the testis hormone in normal males had a suppressing effect upon the growth and development of the testes of young males entirely similar to that exercised by oestrin.

c. Scrotal Function.

Goubaux and Follin (1855) in studying cryptorchidism in horses and man demonstrated that testes retained in the abdominal cavity in the original position below the kidneys, in the iliac fossa or in the inguinal canal do not undergo normal development. Monod and Arthaud (1877) noted that undescended testes showed atrophy of the seminiferous tubules and no production of spermatozoa. Griffiths (1893), by producing experimental cryptorchidism, demonstrated that the normal testis, after replacement in the abdominal cavity, shrunk in size, and failed to produce spermatozoa. Crew (1923) suggested that the cause of the aspermatic condition of the imperfectly descended testis might be explained by assuming that the abdominal temperature is not that at which the final stages of spermatogenesis occur. Moore (1922) produced experimental cryptorchidism in mature rats and showed that the germinal epithelium of the testes underwent degeneration. After sixty-five days (Moore - 1924) testes of experimentally cryptorchid rams showed complete degeneration of the germinal epithelium with the exception

of a single layer of cells next to the basement membrane. The interstitial tissue underwent little change; the cells of Leydig increased slightly in numbers, and the connective tissue increased in prominence.

Fukui (1933) by applying heat to the free surface of the scrotum brought about typical changes of degeneration in the testis. Moore (1924), by applying heat to the scrotal surface of the guinea pig demonstrated that a single ten-minute application of water at 47°C will result in definite degeneration of the seminiferous tubules at the end of two weeks. Moore and Oslund (1924) by insulating the scrotum of the ram produced degeneration of the seminiferous tubules and the absence of spermatozoa in eighty days. Phillips and McKenzie (1934) showed that scrotal insulation of the ram for periods of two weeks or longer caused definite germinal disintegration and the disappearance of spermatozoa in the tubules. Moore (1923, 1924, 1926) demonstrated that testis grafts produce spermatozoa only when transplanted into the scrotum. Moore (1926) showed that full spermatogenic activity follows the replacement of abdominally retained testes in the scrotum. Moore and Quick (1924) and Phillips and McKenzie (1934) show that there is a difference between the scrotal and abdominal temperatures of rabbits, rats, guinea-pigs, and rams varying from 7.3°C to 1.7°C, varying with species and outside or room temperature.

Heller (1929) introduces new evidence for the function of the scrotum in that he finds that sperm from the isolated epididymis of the guinea pig are capable of being activated in saline for about twenty-three days if the epididymis remains

days if the epididymis is raised into the abdominal cavity.

The mechanism by which the scrotum is able to serve as a thermo-regulator was first suggested by Lieben (1908) who carried on a few experiments with the tunica dartos muscle. Lieben studied the contractions of the scrotums of dogs and humans as he stimulated various parts of the body with warm and cold water, and ether. He found the scrotum to be relaxed at uniform room temperature, and that it was divided in half in regard to control by the autonomic nervous system. Crew (1922) observed that the scrotum varied its state of contraction as atmospheric conditions varied, and suggested that the tunica dartos muscle might regulate the activity of the scrotum.

Phillips and McKenzie (1934) found the intact scrotum to be maintained in a state of contraction at low temperatures and to be completely relaxed at high temperatures.

The scrotum undergoes constant adjustment at intermediate temperatures (6.0°C to 24.0°C); the tunica dartos muscle contracts or relaxes as the scrotal temperature is slightly decreased or increased. Isolated strips of the tunica dartos are sensitive to temperature changes, expecially those approximating the normal scrotal temperature range. The effects of pilocarpine and atropine and epinephrine and ephedrine (Phillips and McKenzie 1934) suggest both a sympathetic and parasympathetic motor innervation of the tunica dartos muscle of the ram.

Phillips and Andrews (1935) in studying the development of the thermo-regulatory function of the tunica dartos muscle of the ram present data gathered on animals from three

to twenty-seven weeks of age. They found that isolated strips of the dartos show little sensitivity to temperature change until an age of twelve weeks. The amount of smooth muscle in the dartos increases gradually from birth to about twenty-one weeks of age, and spermatozoa first appear in the testes at twenty-one weeks. In animals gonadectomized earlier than twelve weeks the dartos failed to develop sensitivity to temperature changes, and if castration was performed at twelve weeks of age or later the sensitivity to temperature changes tended to decrease following castration. Injections of the testicular hormone increases the sensitivity of the tunica dartos muscle, and it responded to temperature changes at an earlier period than in the untreated animal. Injections of an unfractionated extract of the anterior pituitary gland resulted in an increased sensitivity to temperature change but no increase in the altitude of contractions.

d. The Effect of the Plane of Nutrition upon the Development of the Testis.

The work of Papanicolaou and Stockard (1920) demonstrated that a sub-optimal diet retards reproductive development in the female rat, and the results of Evans and Bishop (1923) on the same animal substantiate this finding. Hammett's (1926) results with the rat substantiate the previous work and extend them to include the male. Bowditch (1878), Roberts (1878), and Hall (1908) in reporting work with the human (as reported by Hammett), state that a sub-optimal diet retards the development of the genital organs. Hammett (1926) in studying the relation of the thyroid gland

to the growth of the reproductive system concludes that there is no apparent specific relation between the growth of the reproductive system of either sex and thyroid or parathyroid activity, and the retardation in growth of the genitalia resulting after thyroid or parathyroidectomy is attributable to a general metabolic disturbance which results in a condition of essential undernutrition. McKenzie (1928) in studying growth and reproduction in swine concluded from post mortem data obtained that if the gilt be maintained on a low plane of nutrition, body growth and the development of the genital organs are retarded, and sexual maturity is delayed till the animal is eleven to twelve months old. Moore (1932) is reviewing the biology of the testis states that a lack of vitamin E may cause permanent sterility; lack of vitamin B may result in some injury; and lack of vitamin A may result in reproductive failure.

Mason (1933) in studying the effect of various nutritional deficiencies upon the testis found that in inanition there was a moderate sloughing of germ cells and a cessation of spermatogenesis. Recovery required three to four weeks on full feed. A lack of vitamin C brought about similar degeneration in the guinea pig, and a lack of vitamin D had the same effect on the rat. A deficiency of vitamin A brought about severe sloughing in the tubules, but spermatogenesis did not cease. Upon supplying vitamin A, recovery was brought about in sixty to ninety days. A lack of vitamin E resulted in chromolysis of nuclear material, and the formation of abnormal numbers of giant cells. Recovery was not brought about by therapeutic treatment.

Popoff and Okulicheff (1934) find that high levels of protein and of calcium phosphate tend to increase the volume and density of spermatozoa in the ejaculate of the ram.

e. The Effect of Drugs Upon the Production of Spermatozoa.

Fowler's solution, a solution containing potassium arsenite, and frequently used in fitting livestock for show, may have some effect upon fertility. Roberts and Dawson (1935) found that by treating male rabbits with this solution there is a decreased number of offspring, and a greater percentage of young dead at birth. The feeding of arsenic to male rabbits resulted in less semen per ejaculate, and fewer spermatozoa per volume of semen than normal.

V. The Mature Spermatozoa.

a. Morphology and Fertility.

Hammond and Asdell (1926) showed that motility was not a measure of fertility in the rabbit. Fertility decreases from the twentieth to the fortieth day in cases of ligature of the epididymis, while motility is observed as late as the sixtieth day. Williams and Savage (1926-1927) found that in the bull many insignificant factors may hinder the movements of spermatozoa from highly fertile sires, and that diseased and deformed sperms from poor bulls are often highly motile. Williams (1925) in reporting the results of examinations in the spermatozoa of sufficient extent to permanently interfere with fertility. He found that normal animals show fifty to seventy-five abnormalities per one thousand spermatozoa, and that ten to fifteen percent of abnormal spermatozoa in the bull definitely lessen fertility. Williams and Savage (1926-1927) made a statistical study of sperm

head length in bulls and plotted the results in the form of frequency distribution curves. Symmetrical, narrow curves were usually plotted in the cases of poor bulls. Savage (1927) and Savage, Williams and Fowler (1930) find that the coefficiant of variation of frequency distribution curves is not greater than 4:00 in the normal bull, and 6:00 in the stallion. Moench and Holt (1932) find the maximum normal value to be 11.5 in the human.

Meves (1899), according to Cody (1934), found a certain number of abnormal spermatozoa in all species. Broman (1902) observed abnormal spermatozoa in man, and classified abnormalities as double tails, double heads, and abnormally large or small spermatozoa. Moench and Hall (1931) found that in the human, if the number of abnormal sperm heads is greater than twenty percent, the individual is generally of lowered fertility; abnormalities in excess of twenty-five percent result in sterility. Cody (1934) classified sperm abnormalities in the guinea-pig as more than one tail, double heads, irregular size, swollen body, broken tails, and severed heads.

McKenzie and Phillips (1934) studied the morphology of spermatozoa and its relation to fertility in the ram. They found the common sperm abnormalities to be:--loss of tail, curled tail, tapering head, broken neck, small and large heads, enlarged middle piece, middle piece bead, filiform middle piece, double head, double tail, and cytoplasmic extrusion at base of head. Rams showing more than one hundred abnormalities per thousand were found to be of reduced fertility, provided the semen sample had not been taken immed-

iately following a prolonged rest period.

b. Passage Through the Female Reproductive Tract.

Duhrssen (1893) claims to have observed twelve motile spermatozoa in a diseased fallopian tube removed nine days after his patient entered the clinic; according to reports, coitus had not occurred for three and one-half weeks. Hensen (1881), according to Hoehne and Behne (1914), stated that spermatozoa were immotile sixteen hours following copulation in the guinea pig. Sobotta (1895) states that sperm are absorbed in the uterus of the mouse nine to ten hours post-coitus, and Hammond and Asdell (1926) found spermatozoa incapable of fertilization in the rabbit after thirty hours. These workers believe that conditions which prevent intra uterine life of the sperm for longer than thirty hours kill sperm of low vitality before ovulation occurs ten hours after copulation. Hoehne and Behne (1914) demonstrated a small number of motile sperm in the uterus of the rabbit forty-eight hours after coitus, but found none in the fallopian tubes at this time. Anderson (1922), working with the mare, found vigorous spermatozoa in the uterus and tubes seven and one-fourth hours following coitus. Anderson is of the opinion that sperm rarely live longer than forty hours in the female reproductive tract. Pagenstecker (1859), according to Haftman (1933), observed the uterus of the bat to be filled with spermatozoa in January, though ovulation had not occurred. Courrier (1924-1927) found that in some bats copulation took place in the fall and fertilization did not occur until spring. Hartman (1933) believes it doubtful if the spermatozoa survive the winter in the female tract

and believes copulation to be repeated in the spring. Lewis (1911), in data on twenty-five sows, reports that in only three cases did spermatozoa live longer than twenty hours, and found that in one case a small number of motile cells were present at forty-one and one-half hours. It has been stated that the hen lays fertile eggs fourteen days after coitus. Chappellier (1914) demonstrated absolute sterility from seven to eleven days after the removal of the male. Huhner (1928) believes the spermatozoa lose much of their motility within fifteen minutes and are dead within four hours in the vagina of the human. Koehne and Behne (1914) found that in the human, in most cases, spermatozoa are killed after one hour in the vagina and by the end of two or three days in the uterus and fallopian tubes. Runge (1903) in thirty-two observations on seventeen non-gravid women found spermatozoa to be most quickly destroyed in the vagina. In a few individuals live spermatozoa were present in the uterus thirty-six hours post-coitus, but no living cells were observed in the uterus after three days.

Yochem (1929) found a few feeble motile spermatozoa in the uterine horns and oviducts of the guinea pig after forty-one hours. Motile spermatozoa were observed in the horns of the guinea pig uterus thirty-six hours after injecting guinea pig spermatozoa into the uterus with a hypodermic needle when injected at inter-oestrum and forty-one and one-half hours when injected at oestrum. Guinea pig spermatozoa injected into the rat uterus were mobile for eleven hours, and rat spermatozoa injected into the guinea pig uterus were mobile for four and one-half hours as compared to twelve and

one-half hours when injected into the rat uterus.

Leuckart (1853) found guinea pig spermatozoa in the mid region of the fallopian tubes after fifteen minutes. Hensen (1876) demonstrated the presence of sperm in the ovarian bursa of the rabbit after two and three-fourths hours. Kato (1932) found rabbit spermatozoa in the fallopian tubes one hour after copulation. The work of Whitney (1927) showed sperm to be present in the upper ends of the oviducts of the dog eighteen minutes after ejaculation, and at the utero-tubal junction of the same species thirty to fifty seconds after ejaculation (Evans, 1933). Hartman and Ball (1930) observed large quantities of sperm in the rat uterus thirty seconds after ejaculation.

Long and Evans (1932) state that sperm may reach the distal portion of the tube of the mouse within four hours, and Parkes (1930) gives four hours as the interval required for sperm to reach the infundibulum of the rabbit. Lewis (1911) found sperm in the oviduct of the sow seven hours after copulation, while McKenzie (by communication to Phillips, 1935) states that boar sperm reach the middle of the tubes in three and one-tenth hours and the ovaries in five hours.

Quinlan and Mare (1931) found that five to six hours were required for ram sperm to transverse the tubes of the ewe; and Green and Winters (1935) found that the spermatozoa of the ram reach the infundibulum approximately five hours after copulation, depending upon the length of the female tract and the activity of the spermatozoa. Sperm reach the infundibulum of the ewe out of heat nearly as quickly as if

the ewe were in heat, tending to show that during estrum and copulation the genital tract of the female gives no special response which tends to accelerate the advance of sperm. The life of spermatozoa in the ewe is about twenty-four hours.

c. Factors Influencing Activity and Life of Spermatozoa.

Cohn (1918) in extensive studies of the spermatozoa of the sea urchin found that at a temperature of seventy to seventy-three degrees Fahrenheit spermatozoa live six hours, whereas life was observed at the end of twenty-five hours at a temperature of forty Fahrenheit. A lack of oxygen and an increase in carbon dioxide inactivate spermatozoa, and a decrease in hydrogen ion concentration and carbon dioxide, and an increase in oxygen activate sperm. Cohn found spermatozoa to be activated by water in which the eggs of the same or closely related species had been present, and spermatozoon life to be lengthened by the addition of beef broth to sea water. An increase in spermatozoon activity led to a decreased length of life; a pH greater than 9.4 instantly agglutinates spermatozoa of the sea urchin, and a pH of 7.6 tends to inactivate spermatozoa. Cody (1934) demonstrated that the spermatozoa of the guinea pig could be maintained in a living condition for thirty-six hours if held at a temperature a few degrees lower than room temperature, and that if deprived of oxygen, spermatozoa showed no motility after fifteen hours. Spermatozoa not deprived of oxygen showed motility for longer intervals.

Gray (1927), in studies of the spermatozoa of the sea urchin, concludes that the total energy expended during the

life of a spermatozoon, as well as the level of activity exhibited immediately after activation, depends on the degree of dilution of the sperm suspension. This worker believes that the relatively long life of spermatozoa in a concentrated suspension is the result of an incomplete state of activation on the part of each spermatozoa rather than the narcotic effect of accumulated carbon dioxide. Gray (1927) demonstrated that the egg secretions increase the rate at which spermatozoa of the sea urchin absorb oxygen by about three hundred percent in the case of E. esculentus, but have little effect on E. miliaris.

Hartman (1932) reports a pH of seven plus as the optimum H ion concentration for spermatozoa, and that a one percent lactic acid solution is lethal. An alkaline reaction causes a loss of motility, and the optimum temperature for high motility is 35-37°C. 10°C is the optimum temperature for storage. Hartman reports prostatic secretions to be unnecessary for motility and vaginal secretions to be deleterious. Sunlight increases sperm motility and shortens the life of the germ cells. Walton (1933) states that spermatozoon activity ceases below 5°C, but that mammalian spermatozoa will survive short periods of sub-freezing temperatures and regain activity as the temperature is raised. Yamane (1921) and Baker (1930) found glucose solutions buffered with phosphate to be most favorable to the preservation of motility.

Spermatoxins may play an important part in the life of spermatozoa within the female tract. Landsteiner (1899) demonstrated that the immobilization of bull spermatozoa in

the peritoneum of the guinea pig is hastened by the previous injection of bull spermatozoa, and Metchnikoff (1890) found that he could immunize guinea pigs to rabbit spermatozoa by the injection of rabbit testis extracts. Recent work tends to show that after repeated injections of spermatozoa from the same or other species the blood (rat, rabbit, guinea pig, of fowl) develops the capacity of immobilizing, agglutinating, and even dissolving spermatozoa. The animal is in this way rendered sterile.

d. Movements of Spermatozoa.

Reynolds (1916) in studying the movements of human spermatozoa described five types of motion: 1. a rapid vibration of the tail, with the head, middle piece, and forward portion of the tail held in a straight line; 2. slower than the first and the whole tail moving from side to side with a long, slow stroke, while the head and middle piece sway from side to side; 3. a slower motion in which the tail vibrates back and forth and the sperm tend to "bunt" into any small cove; 4. a rotary motion in which the sperm moves with a screw-like motion; 5. a pendulum motion in which all but the tip of the tail seems to lose flexibility, and the remainder of the sperm is thrown back and forth. Reynolds regarded the first three types as normal, and found them progressively as the spermatozoa gradually became exhausted. He classified types 4 and 5 as abnormal, and found types 3 and 5 least common.

Cody (1924) found the movements of guinea pig spermatozoa to correspond to Reynold's type 2 with the exception of a slight rotation. Cody demonstrated that the middle piece

is apparently the motor center of the sperm. If the head is severed from the tail and middle piece, the tail and middle piece continues to swim in a straight line and shows an increase in endurance. An injury to the tail piece results in the cessation of motility and Popa and Marza (1930) are of the opinion that the proximal centriole is the source of the power of motility.

Cody's work (1924) demonstrated that in general spermatozoa tend to swim in a straight line and against a current if it is not too swift. Motility ceases gradually and when once lost is probably never regained. The reduction of motility because of low temperature, or other adverse conditions may be overcome by restoring ideal conditions.

The results of several workers (Lloyd-Jones and Hays, 1918; Parker, 1931; Logg, 1872; and Henle, 1873) show that mammalian spermatozoa have been observed traveling under their own power at from 1 to 3.6 mm. per minute.

Part III

I. The Normal Testis Development of the Bull

The data herein presented was gathered with the intention of determining: (1) the histological changes undergone in the testis of the bull prior to and including sexual maturity and the production of functional spermatozoa; and (2) the relation of the testis to the thermo-regulatory function of the tunica dartos muscle.

Procedure:

Gonadectomies were performed upon bulls ranging in age from 63-450 days; testis blocks were removed and immediately fixed in Bouin's fluid. These were then bleached, dehydrated, embedded in paraffin, sectioned (8-10 micra), and stained with Mayer's Hemalum and Orange G.

Presentation of Results:

The proliferation and development of the germinal epithelium and sex cells of the bull may be divided into two general periods: firstly, a period of gradual development including all processes up to the appearance of secondary spermatocytes; and secondly, a period of rapid proliferation of germ cells culminating in the production of functional spermatozoa.

The first period in this study includes the bulls between the ages of 63 and 104 days. The seminiferous tubules undergo some increase in size during this time, ranging from 58-81 micra in diameter. The centers of the tubules are filled with a homogenous, semi-opaque, colloidal substance, and no lumina are visible. Spermatogonia appear as a loosely-arranged basement row of cells, their nuclei being propor-

tionately greater than the cytoplasm at this time; however, the cytoplasmic content of the cells increases with age. Primary spermatocytes are at first present in small numbers, and increase in frequency in the 104-days male. Secondary spermatocytes and their derivatives are absent; the Sertoli, or nurse cells, are at first difficult to discern, but their delimitation is slowly brought about. The original loose arrangement of the interstitial cells about the seminiferous tubules is replaced by a compact, dense arrangement of the cells of Leydig as sexual maturity is approached.

The seminiferous tubules of bulls between the ages of 142-261 days undergo rapid development. The opaque luminal material undergoes gradual disintegration, becoming more transparent, and definite with regular lumina appearing in the centers of the tubules. The tubules increase in diameter from 81-154 micra during this period, and interstitial tissue becomes closely and densely arranged about them. Primary spermatocytes appear in all tubules, becoming compactly organized and sharply delimited; secondary spermatocytes and spermatids are proliferated in rapid succession. Spermatozoa appear in many of the tubules, but in small numbers at 224 days; and in all tubules in greater number from this period onward. Sertoli cells with maturing spermatozoa attached are now easily distinguishable.

Testis sections prepared from a mature bull (450 days) showed the same general arrangement and content of tubules as those of the 261-day animal; the only difference being that the spermatozoa were present in greater numbers, and the tubule size had undergone some increase, the average

PLATE I - NORMAL TESTICULAR DEVELOPMENT OF THE BULL

photomicrographs - 130 X

Figure 1 - 63 days

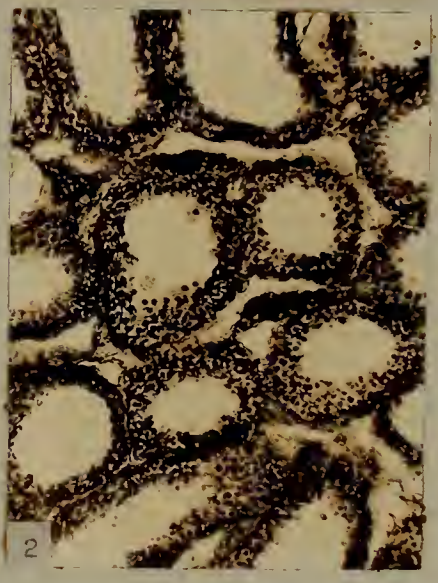


Figure 2 - 224 days

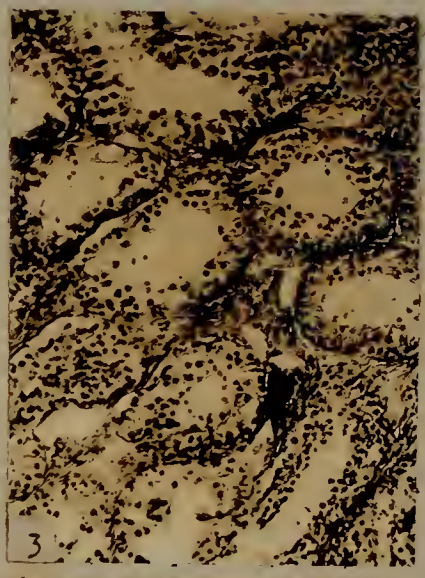


Figure 3 - 261 days

Figure 4 - 450 days

diameter being 217.36 micra.

Photomicrographs showing the changes undergone as sexual maturity is reached are shown in Plate I. In Table I a summary of the development of the seminiferous tubules and interstitial cells is presented.

TABLE I - NORMAL TESTICULAR DEVELOPMENT OF THE BULL

Age Days	Tubule Diameter Micra	Spermatogonia	Sertoli Cells	Primary Spermatocytes	Secondary Spermatocytes	Spermatids	Spermatozoa	Interstitial Cells
63	70.30	Loosely arranged	Indefinite	Very few	None	None	None	Loosely arranged
65	58.05	Loosely arranged	Indefinite	Very few	None	None	None	Loosely arranged
88	60.63	Clearly defined	Indefinite	Few	None	None	None	More densely arranged
104	72.88	Loosely arranged	Indefinite	In most tubules	None	None	None	Densely arranged
142	81.91	Densely arranged	Definite	In all tubules	Few	None	None	Densely arranged
181	116.10	Densely arranged	Definite	In all tubules	In all tubules	Few	None	Densely Arranged
224	148.99	Densely arranged	Definite	In all tubules	In all tubules	In all tubules	In many tubules	Densely arranged
261	154.15	Densely arranged	Definite	In all tubules	In all tubules	In all tubules	In many tubules	Densely arranged
450	217.36	Densely arranged	Definite	In all tubules	In all tubules	In all tubules	In all tubules	Densely arranged

II. The Thermo-regulatory Function and Histological Development of the Tunica Dartos Muscle of the Bull.

The object of the work reported in this section was to study the development of the thermo-regulatory function of the tunica dartos muscle of the bull, and to correlate the sensitivity of this muscle to temperature change with its histological structure and the development of the testis.

Procedure:

The same group of animals used in studying the development of the testis was utilized in this work. A strip of the scrotum was cut from along the longitudinal axis of the pouch; a small section of this was immediately fixed in Bouin's fluid for later histological examination, and the remainder of the strip was prepared for contractility study. The tunica dartos muscle was dissected away from the skin, and mounted in a muscle warmer containing Ringer's solution. One end of the muscle was fixed, and the other attached to a recording lever so that any movements would be recorded on a small drum. Oxygen was passed through the Ringer's solution while the experiment was in progress, and body conditions as to oxygen and osmotic pressure were approximated as closely as possible. The sensitivity and contractability of the dartos was observed over approximately a forty-minute period as the temperature of the Ringer's solution was lowered from 37°C to 20°C, and raised back to 37°C. The dartos strips varied in length from one-half to three-quarters of an inch in length, and movements were magnified approximately eight times on the revolving drum.

The apparatus used in this work is shown in Plate II.

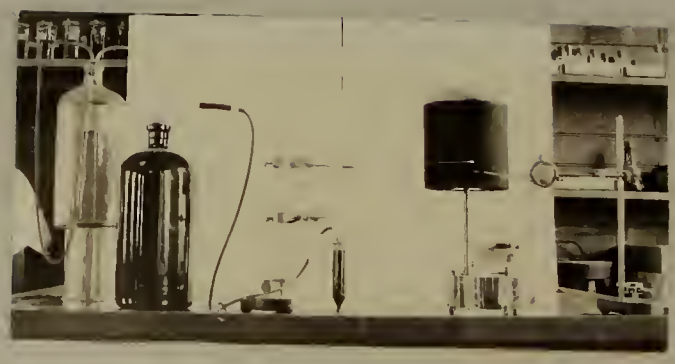


Plate II. Apparatus for studying sensitivity of dartos

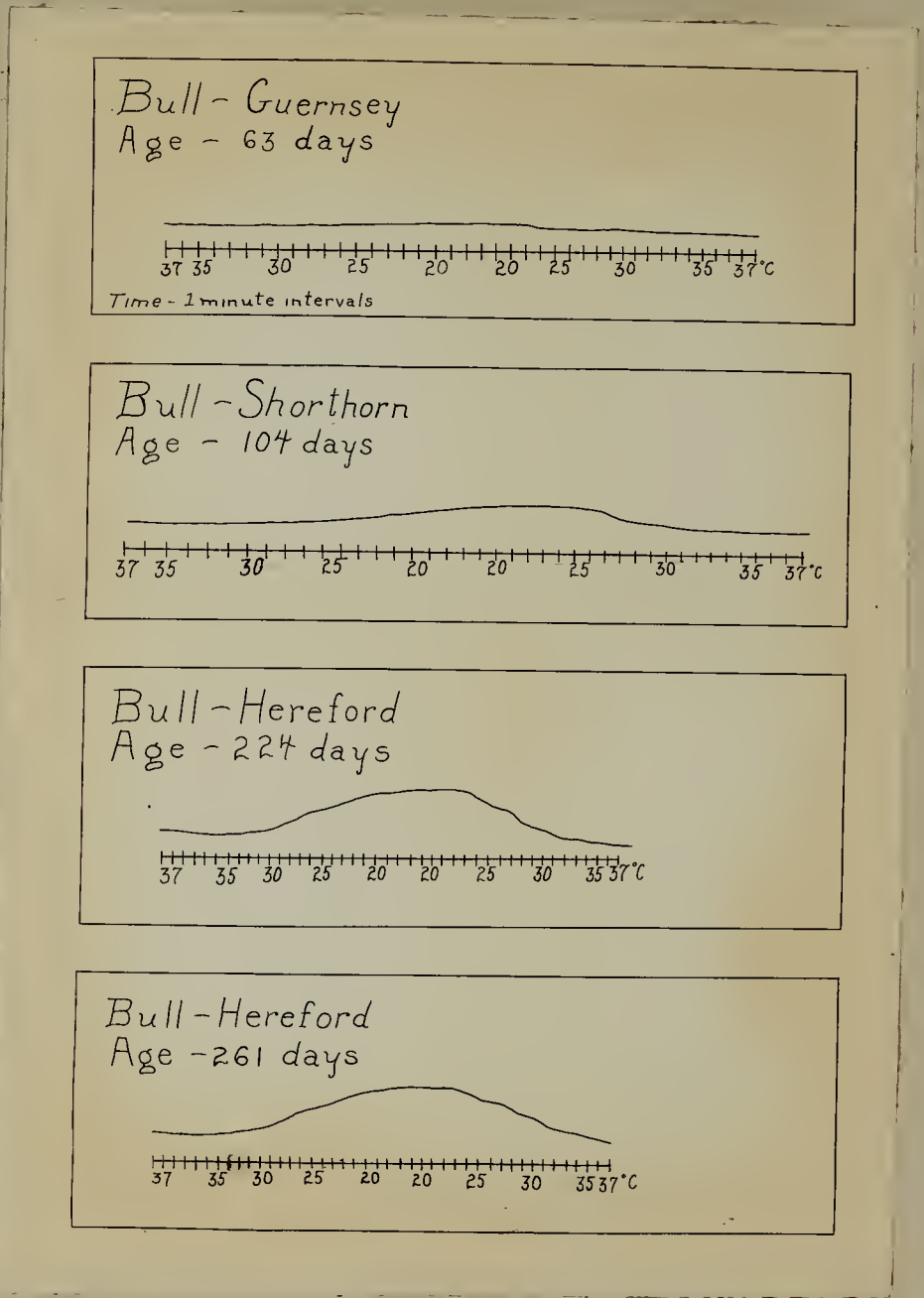
Presentation of Results:

A. Development of Temperature Sensitivity in the Tunica Dartos Muscle.

Dartos reactions of animals ranging in age from 63-261 days indicate that the development of the thermo-regulatory function is a gradual process. Young animals, showing little differentiation of germinal epithelium in the testis, do not possess a high degree of scrotal activity, but as sexual maturity is reached, and mature, functional germ cells are proliferated, the sensitivity and contractability of the tunica dartos muscle undergoes a marked increase. Plate III shows the dartos reactions of bulls of 63, 104, 224, and 261 days respectively, demonstrating the development of the thermo-regulatory function of the dartos.

Table II summarizes the scrotal activity of the males used in this series. The sensitivity and contractability of the muscle increases as the temperature is lowered and the maximum contraction has been measured after maintaining a temperature of 20°C for five minutes. The 88-day Short-

PLATE III - THE DEVELOPMENT OF THE THERMO-REGULATORY FUNCTION OF THE TUNICA DARTOS MUSCLE OF THE BULL



horn bull used in this study shows a greater contraction than any other observed. The testes of this male were apparently of the normal type of an animal of this age, and except for the fact that the bull was in extremely high condition, the author presents no explanation for the scrotal reaction. In all cases except the one previously mentioned, the degree of activity is not marked until sexual maturity is reached (224-261 days in this series).

TABLE II

THE DEVELOPMENT OF SENSITIVITY TO TEMPERATURE CHANGES IN THE TUNICA DARTOS MUSCLE OF THE BULL

Age Days	Temp. at which cont. began (°C)	Altitude of contraction			Maximum contraction mm.	Breed
		30°C	25°C	20°C		
63	36°C	0.2	0.7	1.7	2.0	Guernsey
65	---	---	---	---	0.5	Holstein
88	37°C	11.5	20.0	25.0	27.5	Shorthorn
104	36°C	1.0	3.5	8.0	11.0	Shorthorn
142	36°C	1.5	3.5	6.0	7.0	Hereford
181	32°C	0.5	0.7	1.0	1.0	Hereford
224	36°xC	2.5	13.0	20.5	23.0	Hereford
261	36°xC	3.5	13.5	22.5	24.5	Hereford

B. Histological Changes in the Developing Tunica Dartos Muscle.

The fixed sections of the dartos were bleached, dehydrated, embedded in paraffin, sectioned (8-10 micra), and stained with Mayer's Hemalum and Orange G.

The scrotal wall appears in three distinct layers upon microscopic examination. The first is composed of the skin, with its connective tissue, hair follicles, and sweat and sebaceous glands. The second is made up of loose connective tissue which serves to attach the dartos muscle to the skin, and the third and innermost layer is the tunica dartos itself. The dartos is composed of bundles of smooth muscle fibres freely anastomosing and interspersed with connective tissue. Some muscle fibres on the inner surface run at oblique and right angles to the median line of the scrotum, but the bulk of the smooth muscle tissue is parallel to the long axis of the scrotum.

At 63 and 65 days of age, the bundles of smooth muscle are present as long, narrow strands; the fibres are elongate, spindle-shaped, and contain easily distinguishable nuclei. The general appearance of the scrotal wall is that of a loosely arranged tissue, being made up for the most part of yellow elastic and areolar connective tissue.

The smooth muscle of the dartos undergoes gradual development as sexual maturity is approached. The bundles of muscle fibres become more prominent, the fibres becoming longer and broader with more conspicuous nuclei. The general appearance of the scrotal wall changes; the connective tissue and smooth muscle become more compactly arranged, and at the time of proliferation of spermatozoa the muscle tissue is

PLATE IV - HISTOLOGICAL DEVELOPMENT OF THE TUNICA DARTOS MUSCLE OF THE BULL

photomicrographs - 130 X

Figure 1 - 63 days

Figure 2 - 104 days

Figure 3 - 224 days

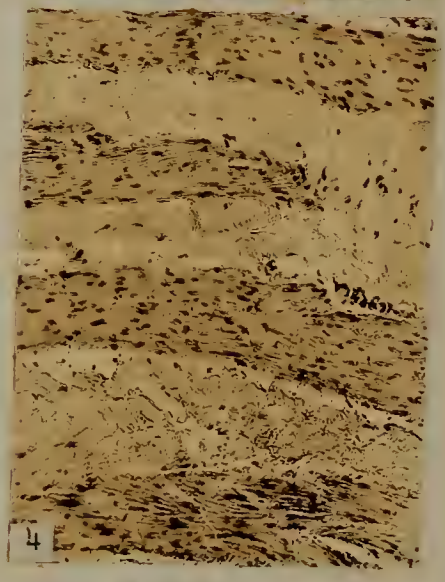


Figure 4 - 261 days

present in large strands.

Photomicrographs showing the development of the tunica dartos muscle appear in Plate IV demonstrating the changes undergone between the ages of 63 and 261 days.

III. Testicular Development of the Boar.

The work described in this section was undertaken with the object of determining: (A) the normal histological changes undergone in the testis of the boar prior to and including sexual maturity; (B) the testicular development of the boar if maintained on a low plane of nutrition.

A. High Plane of Nutrition.

Procedure:

The animals used in this study were weaned at six weeks of age, and from that period onward were allowed free choice of shelled corn, tankage, and pig and hog meal. Each pig received in addition approximately three pounds of skim milk daily. Weekly weight records were kept, and accurate data of the gains from weaning to castration were available. The gonads were excised at intervals between 12 and 147 days, the testicular volume recorded, and sections fixed for histological study by the methods outlined under the development of the testis of the bull. Three of the boars, those 12, 21, and 42 days of age, were castrated during a previous season, and weight records and testicular volumes are not available.

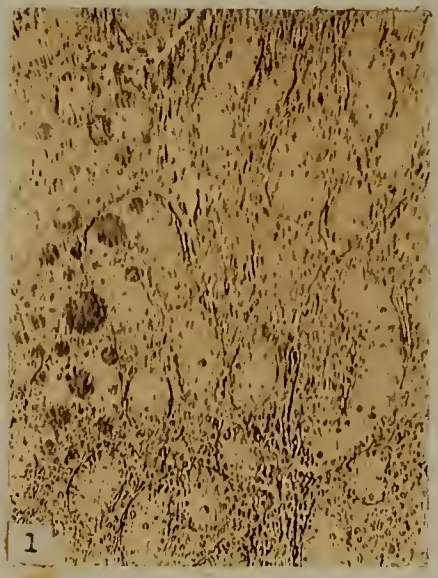
Presentation of Results:

Gonad development in the boar resembles that of the bull in that it may be divided into two general periods. A stage up to and including 84 days during which little development of the germinal epithelium is observed, and a period from 84 days to sexual maturity (147 days) which is marked by the rapid proliferation of all elements of the germinal line including spermatozoa.

Some increase in size of the seminiferous tubules is

PLATE V - TESTICULAR DEVELOPMENT OF THE BOAR -- HIGH PLANE OF NUTRITION

photomicrographs - 130 X



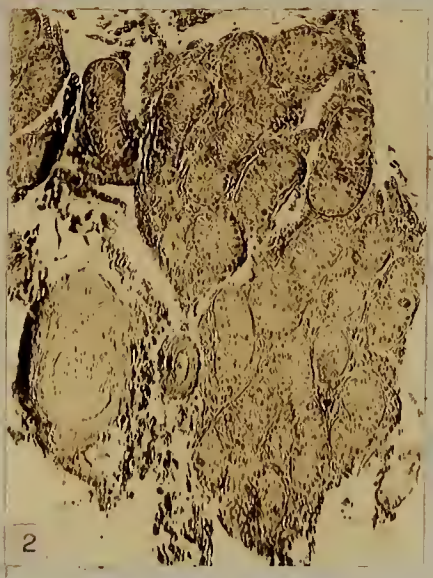


Figure 1 - 61 days

Figure 2 - 84 days

Figure 3 - 105 days

Figure 4 - 147 days

noted prior to 84 days, the average diameter of the tubules ranging between 50-62 micra. The centers of the tubules are filled with a homogenous, semi-opaque, colloidal substance, and no lumina are distinguishable. Spermatogonia, at first loosely arranged in the basement row, become more clearly defined and compactly organized. Primary spermatocytes are proliferated at a low rate, but are observed in all tubules at the end of 84 days. Secondary spermatocytes are lacking, and Sertoli cells are difficult to discern. The interstitial tissue gradually increases in density and compactness of arrangement as the age of the boar increases.

Rapid development is noted in the seminiferous tubules between the ages of 84 and 147 days. The tubule diameter increases from 83-169 micra, and testis volume from 53-150 cc. Regular, clear, lumina appear in the tubules, secondary spermatocytes are produced, and spermatids and spermatozoa are proliferated in rapid succession; mature sperm become distinguishable at 147 days. Sertoli cells are clearly defined, and the interstitial tissue becomes compactly arranged about the seminiferous tubules.

Photomicrographs of testis sections of 61, 84, 105, and 147 days are shown in Plate V, and a summary of the changes undergone between the ages of 12 and 147 days appears in Table III.

TABLE III - NORMAL TESTICULAR DEVELOPMENT OF THE BOAR

Age Days	Tubule Diameter Micra	Spermatogonia	Sertoli Cells	Primary Spermatocytes	Secondary Spermatocytes	Spermatids	Spermatozoa	Interstitial Cells
12	51.60	Loosely arranged	Indefinite	Very few	None	None	None	Loosely arranged
21	50.95	Clearly defined	Indefinite	Very few	None	None	None	Loosely arranged
42	56.76	Clearly defined	Definite	In most tubules	None	None	None	More densely arranged
61	62.56	Clearly defined	Definite	In all tubules	None	None	None	More densely arranged
84	59.34	Densely arranged	Definite	In all tubules	None	None	None	Densely arranged
105	83.85	Densely arranged	Definite	In all tubules	Few	None	None	Densely arranged
126	139.96	Densely arranged	Definite	In all tubules	In all tubules	In all tubules	None	Densely arranged
147	165.76	Densely arranged	Definite	In all tubules	In all tubules	In all tubules	In some tubules	Densely arranged

B. Low Plane of Nutrition.

Procedure:

Five boars, littermates of the animals used in studying the normal development of the testis of the boar, were weaned at six weeks of age and placed on a ration of equal parts of ground oats and middlings fed at the rate of three-quarters of a pound per pig daily. Weekly weight records and gains from weaning to castration were kept. The testes of these animals were removed at 12, 15, 18, 21, and 24 week periods, the volumes recorded, and sections fixed for histological study.

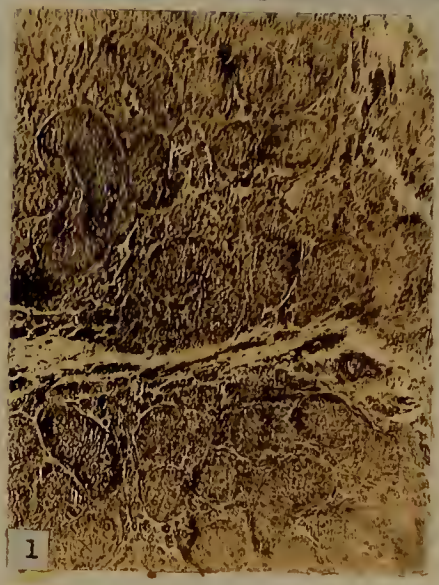
Presentation of Results:

With the exception of the 126-day boar, the results of this study tend to show a definite relationship between the plane of nutrition and the development of the testis. The diameter of the seminiferous tubules increases from 55.47 micra at 84 days to 99.33 micra at 147 days, and testis volume from 5 cc. to 41 cc. during the same interval. This corresponds to a tubule diameter of 165.76 micra and a volume of 150 cc. in a 147-day boar on a high plane of nutrition. The principal changes observed in the testes of boars maintained on a low plane of nutrition were those of arrangement and increase in size of the original elements. The spermatogonia increase in size and compactness of arrangement, and primary spermatocytes are found in about the same frequency in all sections. Secondary spermatocytes are proliferated in the 147-day boar, but are not present in the 168-day animal. Sertoli cells are clearly defined and the cells of Leydig become more densely arranged about the seminiferous tubules.

The testis development of the 126-day boar is far in

PLATE VI - TESTICULAR DEVELOPMENT OF THE BOAR -- LOW PLANE OF NUTRITION

photomicrographs - 130 X



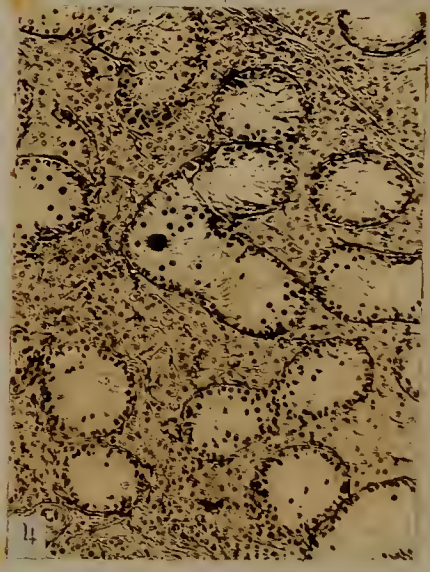


Figure 1 - 84 days

Figure 2 - 105 days

Figure 3 - 147 days

Figure 4 - 168 days

advance of any observed on either the high or low planes of nutrition, and in as much as this boar was apparently of normal physical development, the author advances no theory for the results observed.

Photomicrographs of testis sections of boars of 84, 105, 147, and 168 days are shown in Plate VI, and a summary of testicular development is presented in Table IV.

TABLE IV - TESTICULAR DEVELOPMENT OF THE BOAR ON A LOW PLANE OF NUTRITION

Age Days	Tubule Diameter Micra	Spermatogonia	Sertoli Cells	Primary Spermatocytes	Secondary Spermatocytes	Spermatids	Spermatozoa	Interstitial Cells
84	55.47	Densely arranged	Indefinite	Very few	None	None	None	Loosely arranged
105	58.69	Loosely arranged	Definite	Few	None	None	None	More densely arranged
126	122.55	In all tubules	Definite	In all tubules	In all tubules	In all tubules	In all tubules	Densely arranged
147	99.33	In all tubules	Definite	In all tubules	In all tubules	None	None	Densely arranged
168	96.75	In all tubules	Definite	Few	None	None	None	Densely arranged

Discussion:

Although the amount of experimental data presented is small, there is evidence of an existing relationship between the plane of nutrition and the development of the testis of the boar. Functional spermatozoa are produced in the 21-week boar on a normal plane of nutrition, whereas the secondary spermatocyte is the most advanced germ cell in evidence at 21 weeks on a low plane of nutrition. The 147-day boar on a high plane of nutrition weighed 165 pounds and had a testis volume of 150 cc., as contrasted to a weight of 83 pounds and a volume of 41 cc. in a boar of the same age maintained on a low plane of nutrition.

The testicular volumes and weight records of the boars used in this work are presented in Tables V and VI respectively.

TABLE V - TESTICULAR VOLUMES OF BOARS ON A HIGH AND LOW PLANE OF NUTRITION

Age Days	High Nutrition Group			Low Nutrition Group		
	Hight	Left	Average	Right	Left	Average
61	7 cc.	7 cc.	7 cc.			
84	6	6	6	5 cc.	5 cc.	5 cc.
105	62	45	53.5	6.5	6.5	6.5
126	110	110	110	41	42	41.5
147	150	150	150	42	41	41
168				35	33	34

TABLE VI - WEIGHT RECORDS OF BOARS ON A HIGH AND LOW PLANE OF NUTRITION

Plane of Nutrition	Age Castrated Days	Wt. When Castrated Pounds	Wt. When Weaned Pounds	Gain Pounds
High	61	36	26	10
	84	36	17	19
	105	93	23	70
	126	123	28	95
	147	165	25	140
Low	84	32	18	14
	105	35	19	16
	126	63	27	36
	147	83	27	56
	168	94	23	71

IV. The Thermo-regulatory Function and Histological Development of the Tunica Dartos Muscle of the Boar.

The experimental work conducted in this section was organized for the purpose of studying: (A) the normal development of the thermo-regulatory function of the tunica dartos muscle; (B) the development of the thermo-regulatory function in animals on a low plane of nutrition; and (C) the relation of the sensitivity of this muscle to temperature change to its histological structure and the development of the testis.

Procedure:

The same animals utilized in studying the development of the testis on high and low planes of nutrition were used in this work. Sections of the scrotum were removed, a portion fixed for later histological study, and a strip of the dartos dissected and mounted in a manner similar to that described in the section on the development of the thermo-regulatory function of the tunica dartos of the bull.

Presentation of Results:

A. High Plane of Nutrition:

The development of temperature sensitivity in the tunica dartos muscle of the boar is of the same general type as that previously described in the case of the bull. The first contraction is observed at approximately 36°C, and the maximum contractability is measured at 20°C. The extent of the activity of the muscle is apparently dependent upon the development of the testis. Boars of 12 weeks of age or less show contractions ranging between 5.5 and 12 mm.; testes sections of these animals show the primary spermatocyte to be the highest type of germ cell present. As sexual maturity

PLATE VII - THE DEVELOPMENT OF THE THERMO-REGULATORY FUNCTION OF THE TUNICA DARTOS MUSCLE OF THE BOAR

HIGH PLANE OF NUTRITION

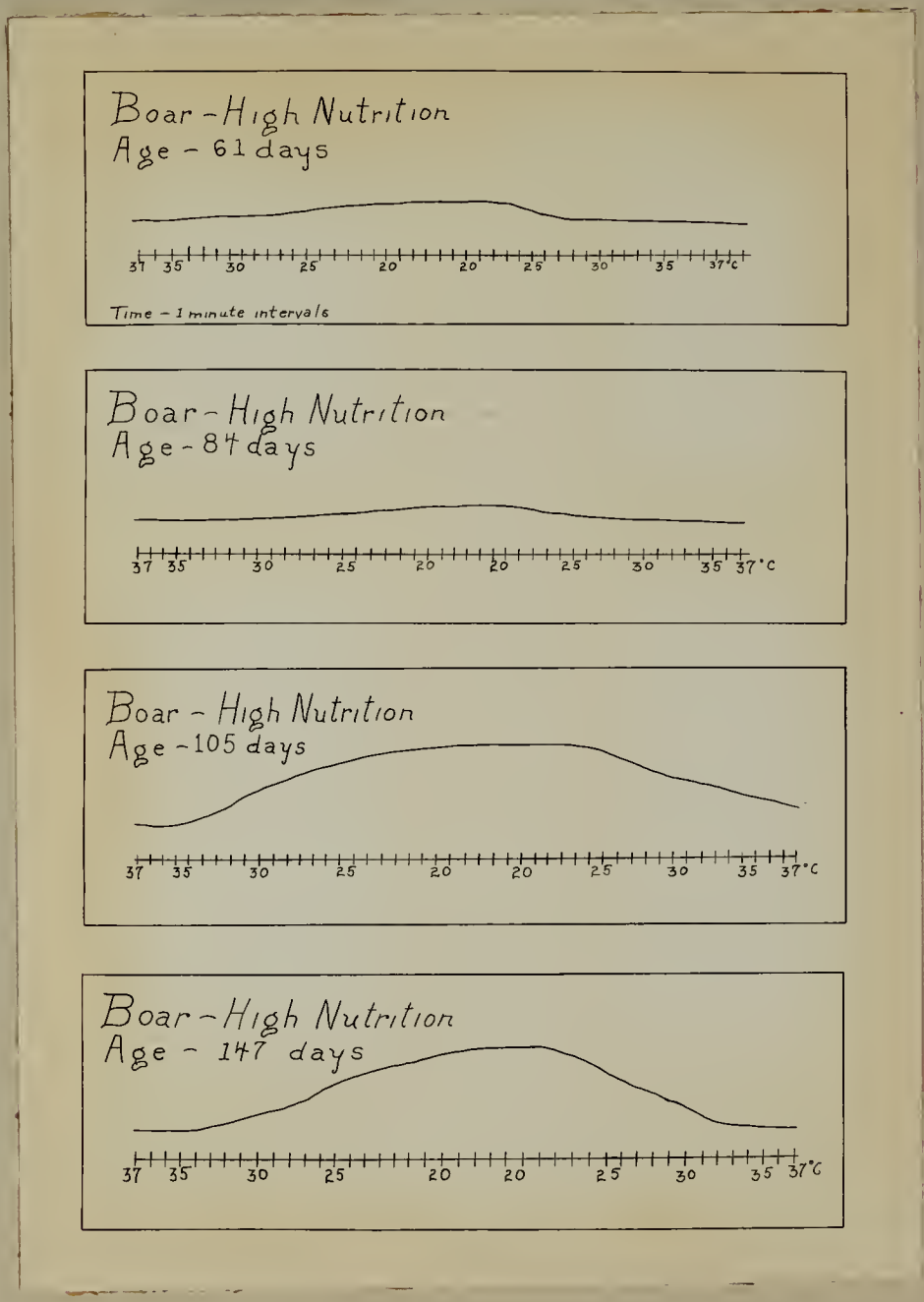


PLATE VIII - THE HISTOLOGICAL DEVELOPMENT OF THE TUNICA DARTOS MUSCLE OF THE BOAR

HIGH PLANE OF NUTRITION

photomicrographs - 130 X

Figure 1 - 61 days

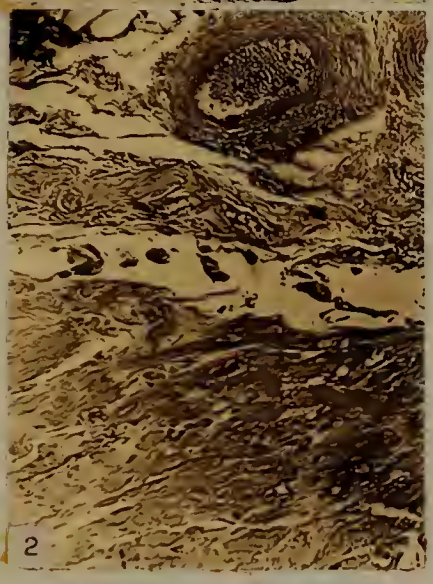


Figure 2 - 84 days

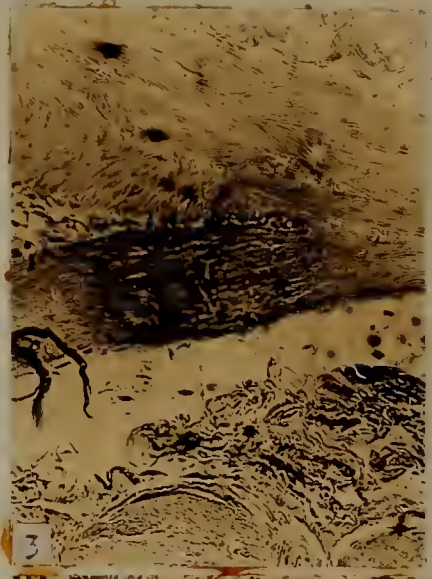


Figure 3 - 105 days

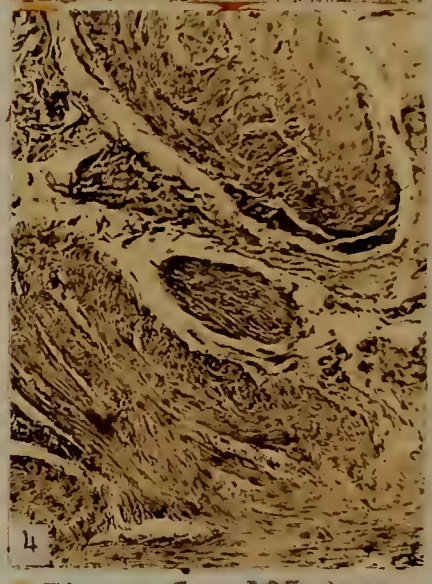


Figure 4 - 147 days

is approached and mature spermatozoa are produced, the height of contraction varies between 41.0 and 68.5 mm. A summary of the scrotal reactions of boars between the ages of 12 and 147 days appears in Table VII, and the development of sensitivity to temperature change is shown in Plate VII.

The histological development of the tunica dartos muscle is also similar to that of the bull. The general appearance of the scrotal wall of a three-weeks boar is that of a loosely-arranged tissue. Smooth muscle is present in narrow, isolated bundles, and the bulk of the section is composed of yellow elastic and areolar connective tissue. As the proliferation of germ cells in the testis increases, there is a corresponding increase in the size of the smooth muscle bundles, and the yellow elastic and areolar connective tissues become more definitely organized.

Photomicrographs of the sections of the dartos of boars of 61, 84, 105, 147 days of age appear in Plate VIII.

B. Low Plane of Nutrition:

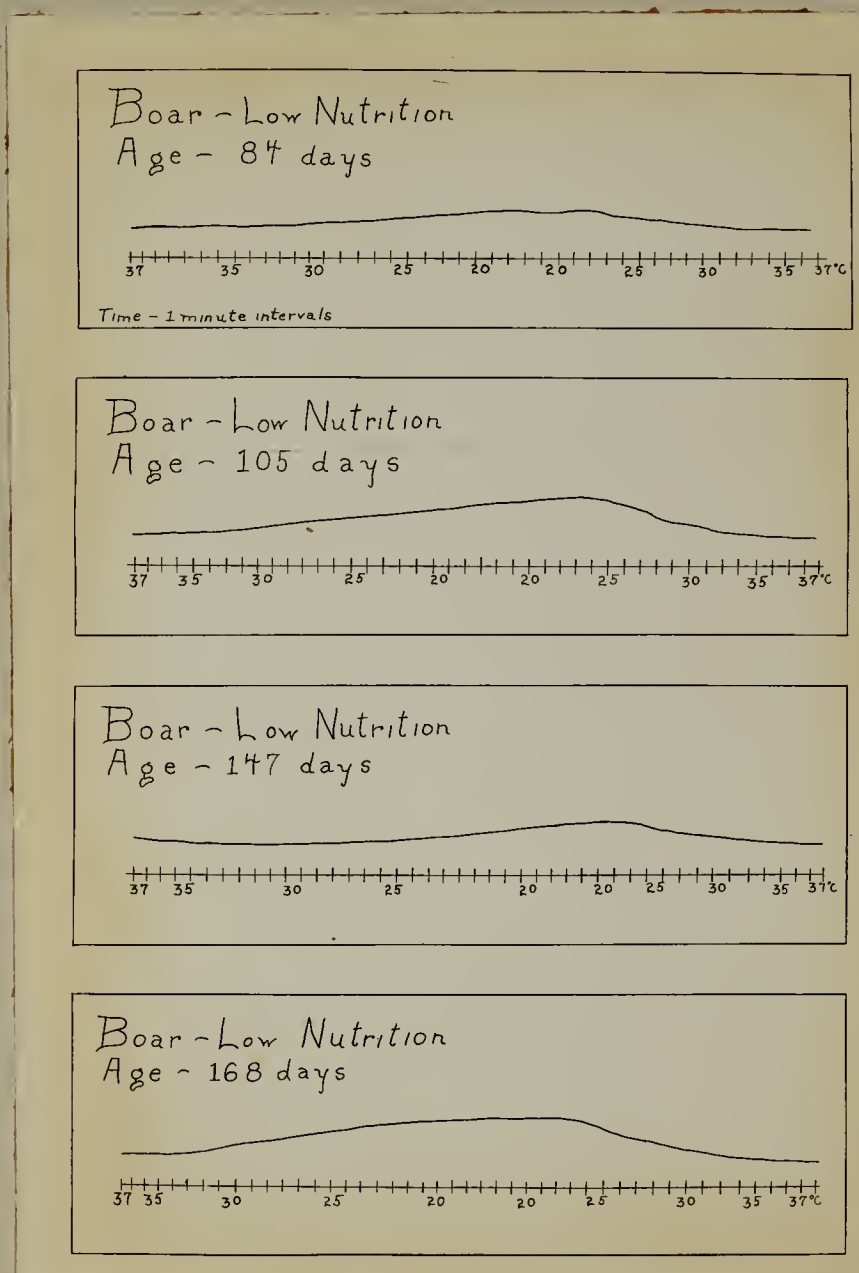
The development of temperature sensitivity is not uniformly brought about in boars maintained on a low plane of nutrition. With the exception of the 126-day boar (described under the development of the testis of the boar on a low plane of nutrition) a high degree of contractility is not manifest, and the maximum scrotal reactions range between 8 and 18.5 mm. as contrasted with 6.5 and 68.5 mm. in the boars on a high plane of nutrition. A summary of the activity of the dartos of these males appears in Table VII, and the development of temperature sensitivity on a low plane of nutrition is presented in Plate IX.

TABLE VII - THE DEVELOPMENT OF SENSITIVITY TO TEMPERATURE CHANGES IN THE TUNICA DARTOS MUSCLE OF THE BOAR

Plane of Nutrition	Age (Days)	Temp. at which contraction began (^{o}C)	Altitude of contraction (mm.)			Maximum contraction (mm.)
			30^{o}	25^{o}	20^{o}	
High	12	37	6.0	9.0	11.0	11.2
	21	36	5.0	9.0	11.5	12.0
	42	36.5	1.0	3.0	5.0	5.5
	61	36	2.2	5.5	9.0	11.0
	84	37	0.5	3.0	6.0	6.5
	105	36	17.5	31.5	39.5	41.0
	126	36	40.0	62.0	67.0	68.5
	147	35.5	7.0	21.0	37.0	41.5
Low	84	34	1.0	4.0	7.0	8.0
	105	36	3.5	8.5	12.5	18.0
	126	36	15.5	31.0	36.0	37.0
	147	31	0.5	2.0	8.5	11.2
	168	36.5	4.5	12.0	17.0	18.5

PLATE IX - THE DEVELOPMENT OF THE THERMO-REGULATORY FUNCTION OF THE TUNICA DARTOS MUSCLE OF THE BOAR

LOW PLANE OF NUTRITION



The histological development of the tunica dartos is of the same general character as that of the boars on a high plane of nutrition. The dartos of the 84-day boar presents the same appearance as that of the male of the same age on a full ration. Conspicuous bundles of smooth muscle are compactly arranged with yellow elastic tissue. The size of the muscle fibres does not undergo as rapid an increase in animals maintained on a low plane of nutrition. The dartos of the 105-day animal does not contain as prominent muscle bundles as that of its littermate of 105 days reported in section A, and the degree of development of the 147-day and 168-day "low" boars corresponds to that of the 105-day "high" male. Smooth muscle is observed in prominent strands, but the relative size of the muscle fibres and bundles resembles that of animals of a lesser age when maintained on a normal ration.

Discussion:

The data gathered in this section of the work shows a relationship between the plane of nutrition, development of the testis, and temperature sensitivity of the tunica dartos muscle. Boars maintained on a high plane of nutrition showed a more rapid development of the germinal epithelium, and a higher degree of sensitivity of the tunica dartos to temperature change. Boars which had reached or were approaching sexual maturity demonstrated the maximum degree of scrotal activity. Males in which sexual maturity had been delayed (low plane of nutrition) did not possess a high degree of scrotal activity.

V. The Transport of Spermatozoa through the Uterine Tubes of the Ewe.

A study of the transport of spermatozoa through the uterine tubes of the ewe was undertaken to determine the length of time required for their passage from the cervix to the infundibulum. The semen was diluted with Ringer's Solution and artificially inseminated. A mixture of ram and rat sperm was used to determine if any difference existed in the speed at which these two types of sperm were transported.

Prodecure:

Four mature Shropshire and three mature Southdown ewes were used in this study. Rat spermatozoa were obtained by macerating the epididymis in four to five cc. of warm Ringer's solution. The spermatozoa of the ram were secured by allowing a male to serve a female, removing the semen from the female by means of a pipette, and immediately mixing two or three cc. of the ram semen with an equal amount of rat sperm in Ringer's Solution. This inseminating mixture of ram and rat spermatozoa was immediately transferred to the experimental female (ewe) by means of a pipette, the sperm solution being left at the cervix of the uterus. The females were inseminated at varying periods, the object being to determine the distance of travel of ram and rat spermatozoa at time intervals ranging from thirty minutes to seven hours. With this in view, the ewes were killed, and the reproductive tracts removed immediately; the connective tissue was dissected away from the Fallopian tubes, which were then straightened out and cut into proximal, medial, and distal sections. The uterine horns were cut into two sections each, and the body of the uterus, the cervix, and the vagina were cut as separate units.

The method employed for determining the presence or absence of sperm consisted of washing each section of the reproductive tract with Ringer's solution from a syringe, beginning first with the distal end of the Fallopian tube, the fimbriated end, and continuing with the remaining sections of the oviduct, uterus, and vagina. The washings were placed on slides, and prepared for microscopic study as follows: the slides were air dried, fixed over a low flame for a few seconds, cleared for thirty minutes in a one percent solution of chlorozene, washed in distilled water, dried, stained for twenty seconds with Ziehl-Neelson's carbol-fuchsin, washed in distilled water, and dried. Ram spermatozoa are characterized by large, oval, symmetrical heads, in contrast to the elongate, spear-shaped heads of rat spermatozoa.

Presentation of Results:

In view of the results secured by Green and Winters (1935) and Quinland and Mare (1931) who found the time of passage of ram sperm from the cervix to the infundibulum to be approximately five hours, the author inseminated the first female for examination seven hours later, the second female at five hours, the third at four hours, the fourth at three hours, the fifth at two hours, the sixth at one hour, and the seventh at thirty minutes.

Ewe 345

This female was inseminated while in oestrus, and killed six hours and fifty-one minutes later. The reproductive organs were removed from the body at exactly seven hours from the time of insemination, and the tract was sectioned into its component parts seven hours and seven minutes from the time

of insemination. An examination of the slides prepared from the tubal washings showed ram spermatozoa to be present in all regions of the tubes, and ram sperm were observed in large numbers at the fimbriated end of the Fallopian tubes.

Rat spermatozoa were observed in all sections, but in very small numbers. Processes of absorption of rat sperm were in progress and the heads of these sperm showed a definite tendency to disappear, leaving the headless tails which were also undergoing degeneration and absorption.

Ewe 700

This ewe was inseminated during the proestrus phase of the oestrus cycle, and her genitalia were removed and ready for examination five hours and twelve minutes from the time of insemination. A study of the slides prepared from this female showed ram spermatozoa to be present at all levels of the reproductive tract. A very few rat sperm, and these undergoing rapid degeneration, were observed in the vagina; none were found in either the uterus or Fallopian tubes.

Ewe 28

Insemination was made in this female during oestrus. The reproductive tract was removed, sectioned, and ready for examination three hours and fifty-nine minutes after insemination. Ram sperm were present on the slides prepared from each section. Spermatozoa of the rat were present in the vagina and the cervical region of the uterus, but were not found in the body of the uterus or the oviducts.

Ewe 27

The suspension of ram and rat sperm was injected into this ewe during proestrus. Her organs were removed, sectioned, and

ready for study three hours and five minutes from the time of insemination. An examination of the slides prepared from the various regions showed ram spermatozoa to be present at the fimbriated end of the Fallopian tubes and all intervening sections. Rat sperm were observed in small numbers in the vagina and the cervix.

Ewe 766

The inseminating fluid was deposited at the cervix, and the reproductive organs ready for examination two hours later. Ram sperm were observed in large numbers in all regions of the female tract. Rat spermatozoa were found in the vagina, uterus, and medial regions of the Fallopian tubes, and had undergone little observable degeneration.

Ewe 608

Insemination was made during proestrus. The animal was killed, and the organs removed and prepared for examination one hour and fourteen minutes later. Ram sperm had completely traversed the tract and were found in large numbers in all regions of the female tract. Rat spermatozoa were found in the vagina, uterus, and medial regions of the Fallopian tubes, and had undergone little observable degeneration.

Ewe 115

This female was inseminated during oestrus and sections were made of her reproductive organs thirty minutes later. Ram spermatozoa were found at all levels of the tract, including the fimbriated end of the tubes. Rat spermatozoa were observed in all regions except the Fallopian tubes.

Discussion:

Summaries of the lengths of the reproductive tracts of the

ewes used in this study and the speed of passage of ram and rat spermatozoa appear in Tables VIII and IX respectively. The rate of travel of ram spermatozoa in the tract of ewe 115 was 12.4 mm. per minute as contrasted with the average speed of 4.83 mm. per minute over approximately the same distance in glass tubes containing Ringer's and NaCl solutions.

Quinland and Mare (1931) and Green and Winters (1935) report the speed of ram sperm to be 1.0-1.3 mm. per minute in the ewe and that 5-6 hours are required by the spermatozoa in reaching the fimbriated ends of the Fallopian tubes. Quinland and Mare state that they found it difficult to determine the presence of sperm, often finding it necessary to examine one hundred fields before distinguishing spermatozoa. The author had no difficulty in detecting the male germ cells, which were present in large numbers in all the experimental animals. Inasmuch as sperm were found in large numbers in all of the ewes studied, the author feels no doubt that spermatozoa can traverse the tract of the ewe in periods less then five hours. It is possible that by inseminating in Ringer's solution the sperm might have been activated to some extent, but in view of the fact that the speed of transport of ram spermatozoa in the ewe (115) is 12.4 mm. per minute, as contrasted to an average time of 4.83 mm. per minute in Ringer's and salt solutions, it is doubtful that such activation should account for the difference in experimental results. Quinland and Mare (1931) and Green and Winters (1935) do not describe the type of service employed, but it is assumed that a normal breeding was used.

From the results obtained it would seem probable that:

(1) The reproductive tract of the ewe is more favorable for the passage of ram spermatozoa than for the germ cells of the male rat.

(2) Ram spermatozoa travel more rapidly in the female tract than over the same distance in vitro.

TABLE VIII - MEASUREMENTS OF THE REPRODUCTIVE TRACTS OF THE EWES USED IN THIS STUDY

Centimeters

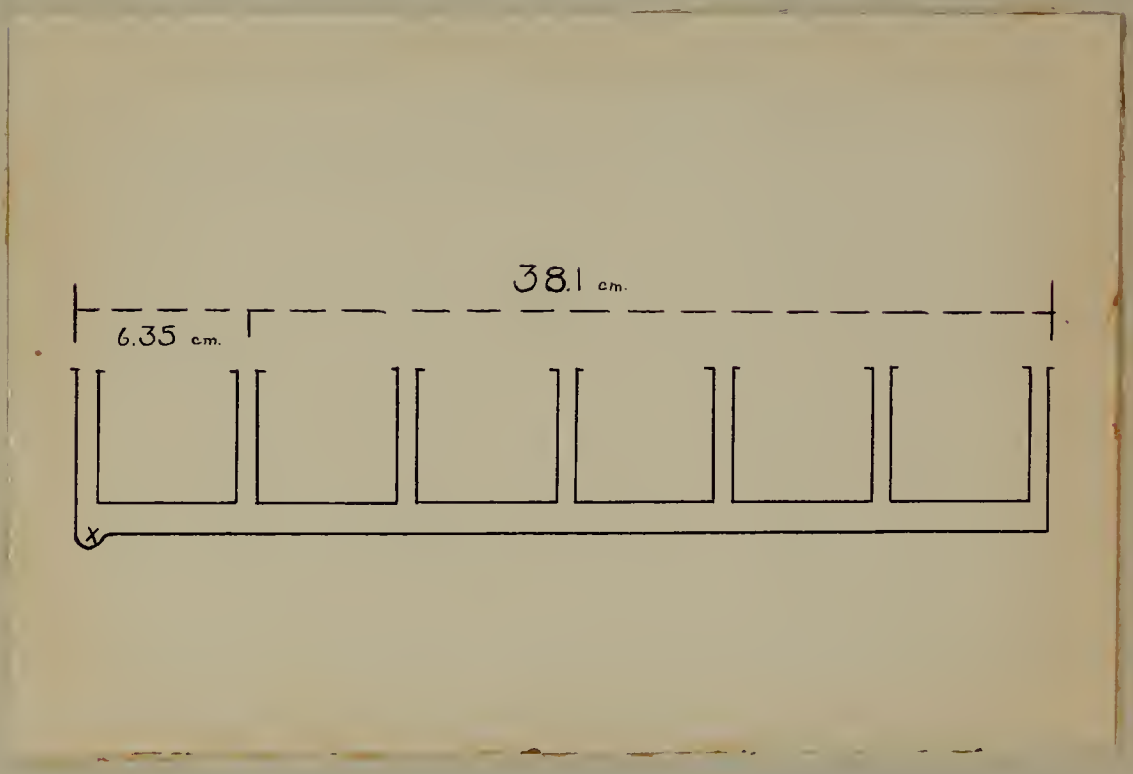
Ewe No.	Vagina	Cervix	Uterus and Uterine horn	Fallopian tubes	Total	Ext. os of Cervix to ovary
115	8.0	6.1	15.2	15.9	45.2	37.2
608	8.5	6.0	17.2	15.6	47.3	38.8
766	10.3	8.2	15.8	17.7	52.0	41.7
27	10.2	7.0	16.0	16.5	49.7	39.5
28	8.2	7.0	14.4	14.8	44.4	36.2
700	8.0	8.7	17.5	17.9	52.1	44.1
345	10.9	7.3	12.0	17.0	47.2	36.3
Average	9.1	7.1	15.4	16.5	48.2	39.1

TABLE IX - SPEED OF TRANSPORT OF RAM AND RAT SPERMATOZOA IN THE REPRODUCTIVE TRACT OF THE EWE

Ewe No.	Time after insemination to sectioning of organs	Ram sperm present at ovary	Rat sperm		Uterus	Tubes
			Vagina	Cervix		
115	30 minutes	yes	yes	yes	yes	no
608	1 hour 14 minutes	"	"	"	"	"
766	2 hours	"	"	"	"	yes
27	3 hours 5 minutes	"	V.F.	V.F.	no	no
28	3 hours 59 minutes	"	yes	yes	"	"
700	5 hours 12 minutes	"	V.F.	no	"	"
345	7 hours 7 minutes	"	V.F.	V. F.	V. F.	V.F.

V.F. = Very few (Some tails present; heads disappear)

PLATE X - APPARATUS FOR DETERMINING THE SPEED OF SPERM TRAVEL IN VITRO



VI. The Speed of Travel of Ram Spermatozoa in Vitro.

Inasmuch as the bulk of experimental work upon the speed of spermatozoan travel in vitro has been carried on by means of the hanging drop and microscopic slide, thus measuring their speed for short distances, the author has undertaken a study to determine the speed of travel of ram spermatozoa in vitro over distances approximating the length of the reproductive tract of the ewe.

Procedure:

Glass tubes 12 mm. in diameter, 38.1 cm. in length, and with seven side arms as shown in Plate X were constructed. Spermatozoa for these studies were obtained by allowing a ram to serve a female, immediately removing the semen from the ewe by means of a glass pipette, and transferring the sperm sample to a glass container where it was held at a temperature of 39°-40°C until placed in the glass tubes a few minutes later. At the time of securing the semen, smears were made, and the numbers and types of abnormal spermatozoa tabulated.

Two solutions, Ringer's and 0.9% NaCl, ranging in pH from 7.6-7.8 were used as sperm media in this study. Baker's solution (glucose-phosphate) was used in preliminary trials, but because of the variation in results, and the difficulty of determining the presence of sperm in this media, it was abandoned.

In order to determine the possibility of currents in the glass tubes, two methods of checking were used. In one, fine granules of carbon were placed in the tubes, and their movements followed. In the other, companion glass tubes were used, and sperm were placed in both. From one tube, samples

were taken at all openings; and from the other, only at the last opening, and then not until the estimated time necessary for the sperm to reach the most distal end of the tube had passed. Thus it was determined that sampling with a pipette at various intervals did not create currents within the glass tubes.

The sperm media were warmed to 39°-40°C and held in a water bath at those temperatures during the entire trial. A drop of semen was placed in the depression marked (x) at one end of the tube. (This depression prevented the flow of semen along the bottom of the tube). Sample drops were removed at regular intervals from the various regions of the tubes, and slides were prepared to determine the presence or absence of spermatozoa.

Presentation of Results:

I. The Rate of Travel of Normal Spermatozoa.

The first section of the work was carried on during the normal breeding season for this species (September-November) and at this time the rate of travel of normal ram spermatozoa was determined. Three rams were utilized during this study; all were in excellent physical condition, and the numbers of abnormal spermatozoa averaged 55 per 1000. The bulk of abnormalities observed were coiled-tails and tailless sperm, small numbers of these types appearing in the semen of all of the farm animals. A fourth ram was used in three trials during February. His semen contained 96 abnormal sperm per 1000.

A. Ringer's Solution.

Eighteen trials conducted with Ringer's solution showed the average time required for ram spermatozoa to traverse the

38.1 cm. glass tube to be 75.27 minutes. Sperm traveled at an average rate of 5.06 mm. per minute in these trials.

B. 0.9 % NaCl Solution.

Spermatozoa traveled at an average speed of 4.72 mm. per minute in seven trials with NaCl solution. The average time required for sperm to reach the distal ends of the tubes was 80.71 minutes.

Summaries of the rate of sperm travel of normal ram spermatozoa in Ringer's and 0.990 NaCl solutions appear in Tables X, XI, XII, and XIII.

II. The Rate of Travel of Abnormal Spermatozoa:

A. Ringer's Solution.

Semen was secured from two rams in February, three months after the normal breeding season, and the types and numbers of abnormal spermatozoa were observed and recorded. Fourteen trials with these rams over a two-week period showed a relation between the morphology of ram spermatozoa and their rate of travel.

Semen containing 972 abnormal sperm per 1000 was used in the first trial, and spermatozoon passage was not observed beyond the third opening in the glass tubes. As the trials progressed, and the numbers of abnormal spermatozoa decreased to 408, 318, and 212 abnormal sperm per 1000, the distance of spermatozoon travel was lengthened, and sperm were finally observed at the distal openings in the tubes. Movements were irregular, and the numbers of spermatozoa appearing in the various regions of the tubes were small.

Summaries of the passage of abnormal spermatozoa in Ringer's solution appear in Table XIV and summaries of the

TABLE X - THE SPEED OF TRAVEL OF NORMAL RAM SPERMATOZOA IN RINGER'S SOLUTION

Tube Opening	Time per Section - minutes					
Ram	2	3	4	5	6	7
I.S.	5	15	50			135
I.S.	12.5	25	30	50	75	105
176	5	10	50	50	90	150
176	3	10	60	60	90	150
176	3	7.5	20	40	50	75
176	5	7.5	20	40	60	75
176	3	5	25	30	30	50
176	3	5	25	30	40	50
176	3	12.5	25			75
174	3	7.5	20	20	40	120
176	3	5	10		25	40
176	3	7.5	20	30	30	60
176						50
176				20	30	50
176	3	7.5	10	15	30	50
172	3	7.5	10	25	40	40
172				25	40	40
172						40
Averages	4.10	9.46	26.78	33.46	47.85	75.27

TABLE XI - THE SPEED OF TRAVEL OF NORMAL RAM SPERMATOZOA IN NaCl SOLUTION

Tube Opening	Time per Section - minutes					
Ram	2	3	4	5	6	7
I.S.	5	15	25	50	75	90
176	5	10	40	50	75	90
176	5	7.5	30	40	50	90
176	5	10			30	75
176	3	7.5	25	40		60
174	3	5	20	25	40	120
176	3	7.5		30	40	40
Averages	4.14	8.92	28.00	39.18	51.80	80.71

TABLE XII - SUMMARY OF SPEED OF TRAVEL OF NORMAL RAM SPERMATOZOA IN RINGER'S AND NaCl SOLUTIONS

Tube Opening	2		3		4		5		6		7	
Solution	Tr	Ti	Tr	Ti	Tr	Ti	Tr	Ti	Tr	Ti	Tr	Ti
Ringer's	14	4.10	14	9.46	14	26.78	13	33.46	14	47.85	18	75.27
NaCl	7	4.14	7	8.92	5	28.00	6	39.18	6	51.80	7	80.71

Tr = Number of trials
Ti = Time in minutes

TABLE XIII - AVERAGE OF RINGER'S AND NaCl SOLUTIONS

Tube Opening	2	3	4	5	6	7
Average time (min.)	4.11	9.28	27.10	35.21	49.03	76.79
Time per section (min.)	4.11	5.17	17.82	8.11	13.82	27.76
Speed per min. (mm.)	15.45	12.28	3.56	7.82	4.58	2.28

Total average speed -- 4.83 mm. per minute

TABLE XIV - THE SPEED OF TRAVEL OF ABNORMAL RAM SPERMATOZOA IN RINGER'S SOLUTION

Tube Opening		Time per section - minutes					
Ram	Abnml sperm per 1000	2	3	4	5	6	7
176	972	3	5	0	0	0	0
176	972	0	0	0	0	0	0
176	408	5	?	10?	0	0	0
176	408	0	0	0	0	0	0
176	No Count	5	7.5	40	0	0	0
176	No Count	0	0	0	0	0	0
176	No Count	5	12.5	12.5	15	60	0
176	No Count	0	0	0	0	0	0
176	318	3	15	20	30	0	0
176	318	0	0	0	20	40	0
176	318	0	0	0	0	0	0
172	212	0	7.5	12.5	0	25	0
172	212	0	0	12.5	0	0	75
172	212	0	0	0	0	0	0

TABLE XV - SUMMARIES OF THE TYPES AND NUMBERS OF ABNORMAL SPERMATOZOA USED IN THESE STUDIES

Average numbers and types of abnormal spermatozoa in semen used in determining the normal speed of travel of ram spermatozoa

Tailless Heads	Coiled Tails	Tapering Heads	Middle-piece Abnormalities	Broken Necks	Total per 1000
25.6	20.8	4	3.8	0.8	55

Numbers and types of sperm abnormalities in semen used in determining the effect of abnormal spermatozoa upon the rate of travel

Ram	Date	Tailless Heads	Coiled Tails	Tapering Heads	Middle-piece Abnormalities	Broken Necks	Total per 1000
176	2/12/36	962	8			2	972
176	2/26/36	352	52		2	2	408
176	2/26/36	350	60			8	318
172	2/28/36	116	84	4	2	6	212

types and numbers of abnormal spermatozoa appearing in the semen used in the "Normal" and "Abnormal" trials are presented in Table XV.

Discussion:

The rate of spermatozoon travel in vitro is apparently closely related to the morphology of the spermatozoa. An average speed of 4.83 mm. per minute over a 38.1 cm. distance was recorded for sperm containing an average of 55 abnormalities per 1000 as compared to a rapid loss of motility and irregular movement of sperm containing from 212-972 abnormalities per 1000. In the trials conducted with normal spermatozoa, although there was no apparent decrease in the percentages of abnormal sperm, the speed of travel increased as the breeding season progressed. It may be that functional activity, unmeasureable by present morphological methods, increases as the males reach the point of maximum breeding efficiency.

Part IV

SUMMARY

I. The Normal Testis Development of the Bull.

The germinal epithelium of the testis undergoes a slow process of development prior to 104 days. Rapid proliferation of all elements of the germinal line occurs between 142 and 261 days; lumina appear in the tubules, secondary spermatocytes and spermatids appear in large numbers, and spermatozoa are produced at 224 days.

II. The Thermo-regulatory Function and Histological Development of the Tunica Dartos Musole of the Bull.

The sensitivity of the dartos to temperature change increases as sexual maturity is approached, and is most marked after the proliferation of spermatozoa. The development of the smooth musculature of the tunica dartos is a gradual process. The bundles of muscle fibres become more prominent as maturity is approached, and are fully developed shortly after mature germ cells appear in the testis.

III. Testicular Development of the Boar.

The testes of boars maintained on a high plane of nutrition undergo gradual development prior to 84 days. In the succeeding stages secondary spermatocytes, spermatids, and spermatozoa appear, functional germ cells being present at 147 days.

Boars maintained on a low plane of nutrition exhibited a retardation in testis development. With one exception, secondary spermatocytes were the highest type of germinal cell, and the difference in testis volumes and tubule diameters of the boars maintained on high and low planes of nutrition was marked.

IV. The Thermo-regulatory Function and Histological Development of the Tunica Dartos Muscle of the Boar.

The development of dartos activity occurs gradually in boars maintained on a high plane of nutrition, and reaches a maximum point at the time of sexual maturity. The histological development is similar to that of the bull, smooth muscle bundles increasing in size until after the production of spermatozoa.

Boars maintained on a low plane of nutrition show a lessened degree of scrotal activity and a slightly lessened amount of smooth muscle tissue.

V. The Transport of Spermatozoa through the Uterine Tubes of the Ewe.

Ram spermatozoa were found at the fimbriated ends of the Fallopian tubes thirty minutes after insemination (an average speed of 12.4 mm. per minute). Rat spermatozoa had reached the ovary two hours after insemination, and were observed to be undergoing rapid degeneration after five hours in the tract of the ewe.

VI. The Speed of Travel of Ram Spermatozoa in Vitro.

Normal ram spermatozoa, averaging 55 abnormal sperm per 1000, traveled at an average speed of 4.83 mm. per minute in Ringer's and NaCl solutions over a 38.1 cm. distance. When semen containing from 212-972 abnormal spermatozoa per 1000 was used, movements were irregular, and there was a rapid loss of motility.

CONCLUSIONS

1. Marked development of the germinal epithelium of the testis of the bull was first observed at 142 days.
2. Spermatozoa were produced at 224 days in bulls of this series.
3. The thermo-regulatory function of the tunica dartos muscle of the bull is most marked as sexual maturity is approached. The development of smooth muscle in the scrotal wall is nearly complete when mature germ cells appear.
4. The testes of boars maintained on a high plane of nutrition underwent rapid proliferation of germinal elements after 84 days and spermatozoa were produced at 147 days.
5. Boars maintained on a low plane of nutrition, with one exception, showed a ratardation in testis development and a failure to produce spermatozoa in 168 days.
6. Boars maintained on a high plane of nutrition showed the greatest sensitivity of the dartos to temperature change at sexual maturi y.
7. Boars maintained on a low plane of nutrition failed to develop a high degree of dartos activity.
8. The histological development of the tunica dartos of the boar is similar to that of the bull, the maximal amount of smooth muscle being present at the time of proliferation of mature germ cells. A low plane of nutrition slightly retards the development of the tunica dartos.
9. In both the bull and the boar there is a relation between the development of the testis and the thermo-regulatory function of the tunica dartos muscle.
10. Ram spermatozoa were observed at the ovary of the ewe 30 minutes after the time of insemination at the cervix of the uterus.

11. Rat spermatozoa are not transported as rapidly as ram spermatozoa in the ewe, and undergo rapid processes of resorption after 5 hours.

12. Normal ram sperm traveled at a rate of 4.83 mm. per minute in vitro in Ringer's and 0.9% NaCl solutions over a 38.1 cm. distance.

13. There is a relation between the numbers of abnormal ram spermatozoa per 1000 and their rate of travel in vitro.

Part V

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